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Treatment of fruits or vegetables with hypersensitive response elicitor to inhibit postharvest disease or desiccation

Abstract

The present invention relates to a methods of inhibiting postharvest disease or desiccation in a fruit or vegetable, either by treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation, or by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant. Also disclosed are DNA constructs and expression systems, host cells, and transgenic plants containing the DNA construct.

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Claims

What is claimed:

1. A method of inhibiting postharvest disease or desiccation in a fruit or vegetable, said method comprising: treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

2. The method according to claim 1, wherein hypersensitive response elicitor protein or polypeptide is in isolated form.

3. The method according to claim 2, wherein said treating is carried out prior to harvest of the fruit or vegetable.

4. The method according to claim 3, wherein said treating is carried out by spraying the fruit or vegetable with the hypersensitive response elicitor protein or polypeptide.

5. The method according to claim 4, wherein the hypersensitive response elicitor protein or polypeptide is in liquid or powder form.

6. The method according to claim 1, wherein said treating is carried out after harvest of the fruit or vegetable.

7. The method according to claim 6, wherein said treating is carried out by spraying the fruit or vegetable with the hypersensitive response elicitor protein or polypeptide.

8. The method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is in liquid or powder form.

9. The method according to claim 6, wherein said treating is carried out by immersing the fruit or vegetable in the hypersensitive response elicitor protein or polypeptide.

10. The method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of Erwinia, Xanthomonas, Pseudomonas, Phytophthora, and Clavibacter.

11. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia amylovora.

12. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia carotovora.

13. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide

is derived from Erwinia stewartii.

14. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia chrysanthemi.

15. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from Pseudomonas syringae.

16. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from Pseudomonas solanacearum.

17. The method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of Phytophthora.

18. The method according to claim 1, wherein said treating inhibits desiccation in a fruit or vegetable.

19. The method according to claim 1, wherein said treating inhibits a postharvest disease in a fruit or vegetable.

20. The method according to claim 19, wherein the postharvest disease is caused by Penicillium, Botrytis, Phytophthora, or Erwinia.

21. A method of inhibiting postharvest disease or desiccation in a fruit or vegetable, said method comprising: providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

22. The method according to claim 21, wherein a transgenic plant is provided.

23. The method according to claim 21, wherein a transgenic plant seed is provided.

24. The method according to claim 21, wherein the transgenic plant is a dicot or a monocot.

25. The method according to claim 21, further comprising: applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to inhibit postharvest disease or desiccation.

26. The method according to claim 25, wherein said applying is carried out prior to harvest of the fruit or vegetable.

27. The method according to claim 25, wherein said applying is carried out after harvest of the fruit or vegetable.

28. The method according to claim 21, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of Erwinia, Xanthomonas, Pseudomonas, Phytophthora, and Clavibacter.

29. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia amylovora.

30. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia carotovora.

31. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia stewartii.

32. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia chrysanthemi.

33. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from Pseudomonas syringae.

34. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from Pseudomonas solanacearum.

35. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of Phytophthora.

36. The method according to claim 21, wherein the postharvest disease is caused by Penicillium, Botrytis, Phytophthora, or Erwinia.

37. A DNA construct comprising: a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide; a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue; and a 3' regulatory region operably coupled to the DNA molecule, wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation.

38. An expression system comprising a vector into which is inserted a heterologous DNA construct according to claim 37.

39. A host cell comprising a heterologous DNA construct according to claim 37.

40. The host cell according to claim 39, wherein the host cell is a plant cell or a bacteria cell.

41. The host cell according to claim 40, wherein the bacteria cell is an Agrobacterium cell.

42. A transgenic plant comprising a heterologous DNA construct according to claim 37.

43. A method of enhancing the longevity of fruit or vegetable ripeness comprising: treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhance the longevity of fruit or vegetable ripeness.

44. The method according to claim 43, wherein hypersensitive response elicitor protein or polypeptide is in isolated form.

45. The method according to claim 43, wherein said treating is carried out prior to harvest of the fruit or vegetable.

46. The method according to claim 43, wherein said treating is carried out after harvest of the fruit or vegetable.

47. A method of enhancing the longevity of fruit or vegetable ripeness comprising: providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to enhance the longevity of fruit or vegetable ripeness in a fruit or vegetable harvested from the transgenic plant.

48. The method according to claim 47, further comprising: applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to enhance the longevity of fruit or vegetable ripeness.

49. The method according to claim 48, wherein said applying is carried out prior to harvest of the fruit or vegetable.

50. The method according to claim 48, wherein said applying is carried out after harvest of the fruit or vegetable.

Description

[0001] This application claims benefit of U.S. Provisional Patent Application Serial No. 60/198,359, filed Apr. 19, 2000, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to methods of treating fruits or vegetables to inhibit postharvest diseases and/or desiccation of harvested fruits or vegetables.

BACKGROUND OF THE INVENTION

[0003] Postharvest diseases are often extensions of disease occurring in the field or orchard. Brown rot of stone fruits (Monilinia fructicola (Wint.) Honey), for example, may cause blossom and twig blighting in the orchard. Infections in the orchard may not be visible at harvest if fruits are not refrigerated. Colletotrichum gloeosporioiders (Penz.) Arx may attack blossoms or leaves and young fruit of citrus, avocados, mangos, papayas, and a wide range of other tropical and subtropical species; infections in developing fruit are usually latent, and rot lesions appear only at the onset of fruit ripening. Pezicula malicorticis (Jacks.) Nannfld. causes cankers of limbs of apples and pears; infections in developing fruit are latent, and active rotting usually commences only after the fruit has spent several months in storage and proceeds during -1.degree. C. storage because the organism is able to grow at very low temperatures. These fungi used as examples are able to penetrate the cuticle and epidermis of the fruit.

[0004] Whether capable of being penetrated directly or not, wounds are often the usual means by which the fungus enters fruit. Cuts, punctures, bruises, and abrasions cannot be avoided completely during harvest and handling. If the cuticle and epidermis are broken, spores find nutrients and humidity in fresh wounds ideal for spore germination and colonization. Separation of fruits from the parent plant at harvest creates an unavoidable wound that encourages stem-end rots.

[0005] Rots developing at the blossom end usually involve prior colonization of floral parts. For example, Botrytis blossom-end rot (B. cinerea) sometimes occurs in Bartlett pears after a month or two in storage at -1.degree. C. Initiation of rot in fruit flesh is associated with old styles and stamens retained within the

fruit. Floral infections occur in the senescing floral parts at the end of blossoming. Mostly these floral parts are invaded by Alternaria spp. and common saprophytic fungi, but B. cinerea also is found occasionally. Not all fruits having B. cinerea-invaded floral parts rot in storage, but a significant percentage do. By contrast, test fruits remain free from Botrytis blossom-end rot if the old floral parts of developing fruits are free from B. cinerea. Rotting of fruits in storage is greatly reduced by a single orchard spray with a fungicide at the end of blossoming.

[0006] Contact infection, by which mycelia grow from a rotting fruit to contact and penetrate nearby fruit, is an especially serious aspect of some very common postharvest pathogens. The ever-enlarging "nest" of rotting fruit tied together by fungus mycelia will involve all fruit in a container, if given sufficient time.

[0007] Disease or threat of disease dictates in large measure the manner in which perishable fruits are handled. In recent decades, fruits have been shipped to increasingly greater distances from points of production. Exploitation of these distant markets, however, may offer large economic benefits only if the life of the commodity is stretched to its limit. Diseases and disorders ordinarily manageable during handling and transcontinental transit and marketing may be excessive when transoceanic marine transport of longer duration is involved. Similarly, the extension of marketing periods by storing fruits until they near the end of their physiological life may cause additional disease problems. Losses are especially serious if they occur in market areas, because the costs of sorting, packaging, cooling, storage, and transportation, which may greatly exceed production costs, have already been incurred. Of even greater long-term importance may be an impaired reputation leading to reduced future sales.

[0008] Postharvest diseases of fruit cause 15 to 25% losses yearly in the fruit industry worldwide and much of this is due to rot caused by microorganisms. Fungicides, which have been the primary means of controlling postharvest diseases, have come under scrutiny as posing potential oncogenic risks when applied to processed foods. Thus, research efforts have been intensified to develop biological control procedures for postharvest diseases of fruits and vegetables that pose less risk to human health and the environment.

[0009] Considerable attention has been placed on assessing the use of antagonistic microorganisms as a viable alternative to the use of synthetic fungicides. Two basic approaches are available for using antagonistic microorganisms to control postharvest diseases. Naturally occurring antagonists that already exist on fruit and vegetable surfaces have been shown to control several rot pathogens on diverse commodities. Alternatively, artificially introduced antagonists have been shown to be effective in biologically controlling postharvest pathogens.

[0010] Since 1983, an explosion of research has occurred in the area of biological control of postharvest diseases by artificially introduced antagonists, mostly on fruit diseases (Janisiewicz, "Biological Control of Diseases of Fruit," In Biocontrol of Plant Diseases II, Mukergie et al. (ed.), CRC Press, Boca Raton, pp. 153-165 (1988) and Wilson et al., "Potential for Biological Control of Postharvest Plant Diseases," Plant Disease 69:375-378 (1985)). For example, rot on apples was controlled with yeast (Wisniewski et al., "Biological Control of Postharvest Diseases of Fruit: Inhibition of Botrytis Rot on Apples by an Antagonistic Yeast," Proc. Electron Microsc. Soc. Am. 46:290-91 (1988)), while brown rot in apricots was controlled with Bacillus subtilis (Pusey et al., "Postharvest Biological Control of Stone Fruit Brown Rot by Bacillus subtilis," Plant Dis. 68:753-56 (1984)). Mold incidence was reduced from 35% to 8% in lemon peel by a species of Trichoderma (De Matos, "Chemical and Microbiological Factors Influencing the Infection of Lemons by Geotrichum candidum and Penicillium digitatum," Ph.D. dissertation, University of California, Riverside, 106 pp. (1983)). Biocontrol of citrus rot pathogens was demonstrated with Bacillus subtilis (Singh et al., "Bacillus subtilis as a Control Agent Against Fungal Pathogens of Citrus Fruit," Trans. Br. Mycol. Soc. 83:487-90 (1984)). Such antagonists have various modes of action: antibiosis or

competition for nutrients and space or both, induction of resistance in the host tissue, and direct interaction with the pathogen (Wilson et al., "Biological Control of Postharvest Diseases of Fruits and Vegetables: An Emerging Technology," Annu. Rev. Phytopathol. 27:425-441 (1989)).

[0011] While treatment with antagonistic bacterial or fungal species may be, at least to some extent, effective in controlling postharvest diseases, there are a number of factors which must be considered before this approach is used in commercial applications. First, the antagonists must be grown and maintained for use in treatments. This may result in significant expense and regulatory burdens depending on when and how frequently such antagonists would be applied. Also, it is questionable whether growers would want to maintain bioreactors for growing and propagating particular antagonist strains. Second, the efficacy of those antagonists may depend on storage conditions during shipment of harvested fruit. Some antagonists may not be able to tolerate variations in conditions during shipment, thereby allowing the pathogens to overcome any inhibitory effects of the antagonists. Given the above problems, it is not surprising that few of the antagonists reported to control plant pathogens have been successfully transferred from the laboratory into the field or postharvest environment.

[0012] Thus, there still exists a need to provide an effective, commercially viable method for treating fruits and vegetables to control postharvest diseases which avoids entirely or otherwise significantly reduces the need for fungicide treatments. In particular, it would be desirable to provide an effective, practicable treatment which presents little or no harm to humans or the environment.

[0013] The present invention is directed to overcoming these and other deficiencies in the art.

SUMMARY OF THE INVENTION

[0014] The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

[0015] A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

[0016] Another aspect of the present invention relates to a DNA construct that includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule, wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation. Also disclosed are expression systems, host cells, and transgenic plants which contain a heterologous DNA construct of the present invention.

[0017] By the present invention, the hypersensitive response elicitor protein or polypeptide can be used to inhibit or otherwise control postharvest diseases (i.e., caused by pathogens) in fruits or vegetables. Likewise, such treatment can also inhibit postharvest desiccation of treated fruits or vegetables. In achieving these objectives, the present invention enables produce growers, warehouse packers, shippers, and suppliers to process, handle, and store fruits and vegetables with reduced losses caused by postharvest disease and desiccation. As a result, the cost of bringing fruits and vegetables from the field to the

consumer can be reduced. Importantly, the quality of the treated fruits or vegetables is improved.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

[0019] A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

[0020] For use in accordance with these methods, suitable hypersensitive response elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

[0021] Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors derived from Erwinia species (e.g., Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora, etc.), Pseudomonas species (e.g., Pseudomonas syringae, Pseudomonas solanacearum, etc.), and Xanthomonas species (e.g., Xanthomonas campestris). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors derived from Gram-positive bacteria. One example is the hypersensitive response elicitor derived from Clavibacter michiganensis subsp. sepedonicus.

[0022] Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitins) from various Phytophthora species (e.g., Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamomi, Phytophthora capsici, Phytophthora megasperma, Phytophthora citrophthora, etc.).

[0023] Preferably, the hypersensitive response elicitor protein or polypeptide is derived from Erwinia chrysanthemi, Erwinia amylovora, Pseudomonas syringae, or Pseudomonas solanacearum.

[0024] A hypersensitive response elicitor protein or polypeptide from Erwinia chrysanthemi has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

1 Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser 1 5 10 15 Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser 20 25 30 Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr 35 40 45 Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu 50 55 60 Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser 65 70 75 80 Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys 85 90 95 Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp 100 105 110 Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln 115 120 125 Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met 130 135 140 Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly 145 150 155 160 Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly 165 170 175 Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu 180 185 190 Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala 195 200 205 Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val 210 215 220 Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp 225 230 235 240 Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys

Asp Gly Trp 245 250 255 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys 260 265 270 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln 275 280 285 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr 290 295 300 Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala 305 310 315 320 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala 325 330 335 Asn Ala

[0025] This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. This Erwinia chrysanthemi hypersensitive response elicitor protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

2 cgattttacc cgggtgaacg tgctatgacc gacagcatca cggtattcga caccgttacg 60 gcgtttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgccgca atccggcgtc 120 gatctggtat ttcagtttgg ggacaccggg cgtgaactca tgatgcagat tcagccgggg 180 cagcaatatc ccggcatgtt gcgcacgctg ctcgctcgtc gttatcagca ggcggcagag 240 tgcgatggct gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg 300 ccgtcggatc ccggcagtta tccgcaggtg atcgaacgtt tgtttgaact ggcgggaatg 360 acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc 420 cgatcattaa gataaaggcg gcttttttta ttgcaaaacg gtaacggtga ggaaccgttt 480 caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgtg 540 ggcatccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca 600 aattacgatc aaagcgcaca tcggcggtga tttgggcgtc tccggtctgg ggctgggtgc 660 tcagggactg aaaggactga attccgcggc ttcatcgctg ggttccagcg tggataaact 720 gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct 780 ggcgcagggg ctgggcgcca gctcgaaggg gctggggatg agcaatcaac tgggccagtc 840 tttcggcaat ggcgcgcagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcggcga 900 tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960 caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020 ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgtcgt ccattctcgg 1080 caacggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggctt 1140 gcagggcctg agcggcgcgg gtgcattcaa ccagttgggt aatgccatcg gcatgggcgt 1200 ggggcagaat gctgcgctga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260 ccgccacttt gtagataaag aagatcgcgg catggcgaaa gagatcggcc agtttatgga 1320 tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380 gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440 cgccagcatg gacaaattcc gtcaggcgat gggtatgatc aaaagcgcgg tggcgggtga 1500 taccggcaat accaacctga acctgcgtgg cgcgggcggt gcatcgctgg gtatcgatgc 1560 ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620 atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680 ttattatgcg gtttatgcgg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740 acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttcctc 1800 gtcgctcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860 cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gccccttag 1920 cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980 gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040 aaaatagggc agtttttgcg tggtatccgt ggggtgttcc ggcctgacaa tcttgagttg 2100 gttcgtcatc atctttctcc atctgggcga cctgatcggt t 2141

[0026] The above nucleotide and amino acid sequences are disclosed and further described in U.S. Pat. No. 5,850,015 to Bauer et al. and U.S. Pat. No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

[0027] A hypersensitive response elicitor protein or polypeptide derived from Erwinia amylovora has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

3 Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser 1 5 10 15 Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln 20 25 30 Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn 35 40 45 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met 50 55 60 Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu 65 70 75 80 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu 85 90 95 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr 100 105 110 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro 115

120 125 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser 130 135 140 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln 145 150 155 160 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly 165 170 175 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu 180 185 190 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly 195 200 205 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly 210 215 220 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu 225 230 235 240 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln 245 250 255 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln 260 265 270 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe 275 280 285 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met 290 295 300 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro 305 310 315 320 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser 325 330 335 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn 340 345 350 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn 355 360 365 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp 370 375 380 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu 385 390 395 400 Gly Ala Ala

[0028] This hypersensitive response elicitor protein or polypeptide has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100.degree. C. for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially no cysteine. The hypersensitive response elicitor protein or polypeptide derived from Erwinia amylovora is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia amylovora," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

4 aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa 60 gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct 120 atcggcggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg 180 ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg 240 gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcggtgg tgggctgatg 300 ggcggtggct taggcggtgg cttaggtaat ggcttgggtg gctcaggtgg cctgggcgaa 360 ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa 420 ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct gggtattaac 480 tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac 540 ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggtgatggg 600 caagatggca cccagggcag ttcctctggg ggcaagcagc cgaccgaagg cgagcagaac 660 gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag 720 ctccttggca acgggggact gggaggtggt cagggcggta atgctggcac gggtcttgac 780 ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag 840 ttaggtaacg ccgtgggtac cggtatcggt atgaaagcgg gcattcaggc gctgaatgat 900 atcggtacgc acaggcacag ttcaacccgt tctttcgtca ataaaggcga tcgggcgatg 960 gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac 1020 cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc 1080 aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140 atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200 ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260 cttggcaagc tgggcgcggc ttaagctt 1288

[0029] The above nucleotide and amino acid sequences are disclosed are further described in U.S. Pat. No. 5,849,868 to Beer et al. and U.S. Pat. No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

[0030] Another hypersensitive response elicitor protein or polypeptide derived from Erwinia amylovora has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

5 Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu 1 5 10 15 Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser 20 25 30 Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu